CLAIMS

[Claim(s)]
[Claim 1](a) A neutral solution and a nucleic acid unity solid phase carrier which contain a chaotropic material in a sample containing ribonucleic acid are mixed, After making ribonucleic acid adsorb on a carrier, a solution which contains a chaotropic material if needed washes, (b) An extraction method of ribonucleic acid making ribonucleic acid eluted in an eluate from this carrier by a penetrant remover which consists of water or low-salt concentration buffer solution washing a carrier to which ribonucleic acid stuck, and heating after contacting (c) this carrier into an eluate which consists of water or low-salt concentration buffer solution.
[Claim 2]An extraction method of the ribonucleic acid according to claim 1 a nucleic acid unity solid phase carrier's being a carrier containing a superparamagnetism metallic oxide, and including further a process of separating a nucleic acid unity solid phase carrier and the liquid phase using magnetism.
[Claim 3](a) A neutral solution which contains guanidine salt of 4-7M, 0 to 5% of nonionic surfactant, EDTA of 0 - 0.2mM, and a reducing agent of 0-0.2M in a sample containing ribonucleic acid, and a nucleic acid unity solid phase carrier are mixed, Make ribonucleic acid adsorb on a carrier, (b) Rank it second, and Guanidine salt of 4-7M, The 1st penetrant remover containing 0 to 5% of nonionic surfactant washes a ribonucleic acid-carrier complex, (c) The 2nd penetrant remover that consists of water or low-salt concentration buffer solution of 100 or less mM washes a ribonucleic acid-carrier complex further, (d) An extraction method of ribonucleic acid making ribonucleic acid eluted in an eluate from the above-mentioned carrier by heating after contacting the above-mentioned carrier at the last into an eluate which consists of water or low-salt concentration buffer solution of 100 or less mM.
[Claim 4]Guanidine salt of 4-7M, 0 to 5% of nonionic surfactant, EDTA of 0 - 0.2mM, A neutral solution, a nucleic acid unity solid phase carrier containing a reducing agent of 0-0.2M, A reagent kit for extraction of ribonucleic acid containing an eluate which consists of the 2nd penetrant remover that consists of the 1st penetrant remover, water, or low-salt concentration buffer solution of 100 or less mM containing guanidine salt of 4-7M, and 0 to 5% of nonionic surfactant and water, or low-salt concentration buffer solution of 100 or less mM.

[Translation done.]

DETAILED DESCRIPTION

[Detailed Description of the Invention]
[0001]
[Field of the Invention]This invention relates to the method of extracting ribonucleic acid with simple and sufficient reproducibility using a nucleic acid unity solid phase carrier, and the reagent kit for it from the sample containing ribonucleic acid in more detail about the extraction method of ribonucleic acid, and the reagent kit for it. This invention can be applied also to automatic nucleic acid extractor.
[0002]
[Description of the Prior Art]Since ribonucleic acid (RNA) is a biogenic substance by which the cell and living thing which contain it with the deoxyribonucleic acid (DNA) are characterized, the detection and analysis are one of the genetic engineering technique most important now. Although northern blot analysis has been conventionally used as detection of this ribonucleic acid, and an analysis means, By having developed the reverse transcription polymerase chain reaction (RT-PCR) method which amplifies this DNA after changing RNA into DNA in recent years, It became possible to detect and analyze very a small amount of RNA of a grade undetectable in northern blot analysis promptly and comparatively simple. This is enabled to process many samples at once, and especially in the field of today's clinical laboratory test, it is applied as a retroviral (RNA virus) detecting method, and has become diagnosis of an infectious disease with indispensable art.
[0003]While such detection art progresses, a method of extracting a little ribonucleic acid with promptly simple still more sufficient reproducibility from a biomechanical material is desired strongly. When seeing the existence of the infectious virus in the blood for blood transfusion especially, an extraction method automatable also from the field of the safety under extract operation is desired also from the number of processing.
[0004]In order to extract the ribonucleic acid contained in living thing material generally, it is necessary to destroy first the cell containing ribonucleic acid or virion, and ribonucleic acid serves as a mixture with protein, lipid, sugar, a deoxyribonucleic acid, etc. in the stage. Since ribonucleic acid is easily decomposed by RNase which exists universally in a living body, this destructive reaction (lytic reaction) is performed in the powerful protein modifier which can usually carry out denaturation inactivation of RNase which is protein. Therefore, in order to extract the ribonucleic acid with which an enzyme reaction can be presented eventually, it is also important to remove this protein modifier thoroughly in addition to separation with protein, lipid, sugar, a deoxyribonucleic acid, etc. which are intermingled. Organic solvents, such as phenol, extract as a method used for these separation.
Then, there are a method of carrying out curing salting of the nucleic acid with alcohol (alcoholic precipitate) and a method of collecting nucleic acid after sticking to solid phase specifically using a nucleic acid unity solid phase carrier.

[0005]As the former method, it is AGPC (Acid Guanidine Phenol Chlorohorm). The method [Analytical Biochemistry 162 and 156-159] (1987) is generally used widely. This method by processing (1) biomechanical material in the acidic solution containing guanidine thiocyanic acid, phenol, and chloroform, Perform denaturation of the living body structural failure and protein, and centrifugal separation distributes protein and the deoxyribonucleic acid which carried out (2) denaturation to the intermediate phase of an organic solvent phase and an organic solvent phase, and the aqueous phase, (3) isolating only ribonucleic

acid preparatively -- (4) -- it is the method of collecting as a pellet the ribonucleic acid which was made insolubilizing ribonucleic acid (isopropanol sedimentation method), and was insolubilized by centrifugal separation at the (5) last, by adding isopropanol to this aqueous phase.

[0006]There is the strong point in which ribonucleic acid with special equipment unnecessary [this AGPC method] as compared with the ribonucleic acid extraction method using other ultracentrifugation and high purity which hardly contains a deoxyribonucleic acid is obtained. However, the step which must use phenol and chloroform which are poisons and powerful drugs on the other hand, and requires centrifugal separation, the complicated operation of moving of the aqueous phase, and a long time called an isopropanol sedimentation method is required. Therefore, when many samples, such as a clinical diagnosis, need to be analyzed promptly, simpler and the method of extracting ribonucleic acid in a short time are required.

[0007]On the other hand, the method of extracting nucleic acid from a biomechanical material simpler is reported by Boom and others, using a nucleic acid unity solid phase carrier and chaotropic agent, such as a silica particle, as a method of solving these problems. [J. Clin.Microbiol. and 28(3)495-503(1990)] . This method to (1) biomechanical material A guanidine thiocyanate, EDTA, The solution and nucleic acid unity solid phase (silica particle) which consist of the triton X-100 are mixed, After making nucleic acid stick to this solid phase, the solid phase which (2) nucleic acid combined is separated from the liquid phase, (3) the penetrant remover containing a guanidine thiocyanate washes this solid phase -- (4) -- this solid phase with a 70% ethanol solution next, [wash and] (5) After acetone washes this solid phase further, it is the method of drying solid phase with heating, and nucleic acid being eluted from this solid phase in an eluate at the (6) last, and collecting nucleic acid. The point that nucleic acid can be extracted without [without the strong toxic reagent of phenol etc. is used for this method, and] performing complicated operation of separation of the organic solvent phase by centrifugal separation and the aqueous phase, moving of the aqueous phase, etc. is the feature. nucleic acid is direct in recovering liquid (extract), without performing demineralization by alcoholic precipitate etc., and centrifugal thickening operation, since it is collected in the eluate which consists of a little water or low-salt concentration buffer solution eventually -- it can use for the next enzyme reaction.

[0008]However, generally as for the method of making nucleic acid stick to a carrier using a nucleic acid unity solid phase carrier and chaotropic agent, such as not only the method of this Boom and others but silica, and extracting, the following faults and a problem exist. the process (adsorption process) to which the extraction process by this method makes nucleic acid mainly stick under (1) chaotropic-agent existence at a silica particle, and (2), in order to remove the impurity united nonspecific and chaotropic agent, It consists of 3 of a process (elution process) processes of making nucleic acid eluted from a silica particle with the process (washing process) of washing the silica to which nucleic acid stuck with the penetrant remover and (3) water, or low-salt concentration buffer solution. The chaotropic agent is melted as a penetrant remover of (2), and further, in order to prevent the elution from the silica particle of the nucleic acid at the time of washing, a water soluble organic solvent especially the water which contains ethanol at a rate of about 50 to 80%, or low-salt concentration buffer solution is used from the former here.

[0009]However, since an enzyme reaction is checked when carrying out enzyme treatment of the extract when this water soluble organic solvent remains at the process of (3), after the solution containing ethanol washes, ethanol or volatile, still higher acetone usually washes 100% if needed.

Then, removing the organic solvent thoroughly from a system by stoving is performed.

In order that this desiccation leads to remains of ethanol, and it not only requires a long time, but nucleic acid may combine it with a carrier too much firmly when excessive if its drying time is insufficient, it is known that elution will become difficult and will lead to the fall of a nucleic acid recovery amount or the fall of reproducibility as a result. Thus, the use of an organic solvent not only cannot discern the grade of the desiccation easily, but since organic solvents, such as ethanol and acetone, have inflammability and volatility, when especially hands off operation is considered, danger, such as outbreak of fire, is considered. Then, the method which conquered these problems is needed.

[0010]

[Problem(s) to be Solved by the Invention]The purpose of this invention is to provide the method of extracting ribonucleic acid with still more safely and sufficient reproducibility for a short time, and the reagent for it simple, without using an organic solvent from living thing material.

[0011]
[Means for Solving the Problem]This invention persons pay their attention to an extraction method of nucleic acid using a nucleic acid unity solid phase carrier as an extraction method of ribonucleic acid, After making it stick [in the case of ribonucleic acid] to a carrier unlike a deoxyribonucleic acid as a result of inquiring wholeheartedly, in order to solve the above-mentioned problem, even if low-salt concentration buffer solution which is not included at all washes organic solvents, such as ethanol, It did not elute from a carrier easily but found out that an elution was promoted for the first time by heating. This invention is completed based on this new knowledge.
[0012]Namely, this invention mixes a neutral solution and a nucleic acid unity solid phase carrier which contain a chaotropic material in a sample containing (a) ribonucleic acid, After making ribonucleic acid adsorb on a carrier, a solution which contains a chaotropic material if needed washes, (b) By a penetrant remover which consists of water or low-salt concentration buffer solution washing a carrier to which ribonucleic acid stuck, and heating, after contacting (c) this carrier into an eluate which consists of water or low-salt concentration buffer solution, It is an extraction method of ribonucleic acid making ribonucleic acid eluted in an eluate from this carrier.
[0013]This invention Guanidine salt of 4-7M, 0 to 5% of nonionic surfactant, EDTA of 0 - 0.2mM, a neutral solution containing a reducing agent of 0-0.2M, A nucleic acid unity solid phase carrier, guanidine salt of 4-7M, the 1st penetrant remover containing 0 to 5% of nonionic surfactant, It is a reagent kit for extraction of ribonucleic acid containing an eluate which consists of the 2nd penetrant remover that consists of water or low-salt concentration buffer solution of 100 or less mM and water, or low-salt concentration buffer solution of 100 or less mM.
[0014]
[Embodiment of the Invention]The samples containing the ribonucleic acid in this invention are biomechanical materials, such as a blood serum, plasma, blood, urine, saliva, and body fluid. In addition to the ribonucleic acid of foreign parasite origin, such as a virus, bacteria, or a fungus, with ribonucleic acid, the ribonucleic acid of the internality originating in the living thing which produces these biomechanical materials may also be included.
[0015]The neutral dissolution adsorption liquid and the nucleic acid unity solid phase carrier which contain a chaotropic material in the sample containing ribonucleic acid are added, it mixes, and ribonucleic acid is made to adsorb on a carrier in the 1st process first in this invention.
[0016]pH six to 8 neutral solution containing a chaotropic material is used for the dissolution adsorption liquid used in this invention. . As [know / generally / as a chaotropic material / here / as a chaotropic material] Chaotropic ion (univalent negative ion with a big ion radius) is generated in solution, and it has the operation which makes the water solubility of a hydrophobic molecule increase, and if it contributes to adsorption to the solid phase carrier of nucleic acid, it will not be limited in particular. Although a guanidine thiocyanate, guanidinium hydrochloride, sodium iodide, potassium iodide, sodium perchlorate, etc. are mentioned, specifically, The use of the large guanidine thiocyanate of the inhibition effect or guanidinium hydrochloride to RNase which decomposes ribonucleic acid is [among these] the most preferred. When it changes with chaotropic materials used, for example, uses a guanidine thiocyanate, the ranges of it are 3-5.5M, and as for the operating concentration of these chaotropic materials, when using guanidinium hydrochloride, it is preferred to use it by more than 5M.
[0017]Dissolution adsorption liquid may be made to contain a surface-active agent in order to denature the protein contained in destruction or the cell of a cell membrane. As this surface-active agent, especially if generally used for the nucleic acid extraction from a cell etc., will not be limited, but specifically, Anionic surfactants, such as nonionic surfactants, such as a Triton series surfactant and the Tween system surface-active agent, and N **RAU roil sarcosine sodium, are mentioned. Especially in this invention, it is preferred to use a nonionic surfactant so that it may become 0.1 to 2% of range. It is preferred to make dissolution adsorption liquid contain reducing agents, such as 2-mercaptoethanol or dithiothreitol, in order to denaturalize and deactivate the protein contained in a sample, especially RNase.
[0018]If it is a solid which has a hydrophilic surface which can hold nucleic acid by adsorption, i.e., reversible physical association, under existence of chaotropic ion as nucleic acid unity solid phase used in this invention, it will not be limited in particular. Specifically, a silicon dioxide, i.e., silica, is used preferably. A complex with what performed the surface treatment by chemical modification, and other substances, such as a superparamagnetism metallic oxide, is also contained in other substances which use a silicon

dioxide as the main ingredients, for example, glass, diatomaceous earth, or these. When performing a surface treatment by chemical modification, it may be made tinged with a moderate positive electric charge to such an extent that the reversible combination with nucleic acid is not barred.

[0019]Although particles, a filter, a reaction vessel, etc. are concretely mentioned as a gestalt of these nucleic acid unity solid phase, it is not limited in particular. The gestalt of the grain child in consideration of the efficiency of adsorption and elution is [among these] more preferred. 1-100-micrometer 1-10 micrometers 0.05-500-micrometer are especially preferably more preferred for the particle diameter in that case.

[0020]In the process which makes nucleic acid adsorb on a solid phase carrier in this invention, when carriers are particles, it is preferred to stir with a vortex mixer etc. and to distribute a solution and a carrier uniformly. Here, when the deoxyribonucleic acid is contained with ribonucleic acid in the sample, both nucleic acid of both may be the conditions combined with a carrier. After making it join together, it is preferred to wash the carrier which nucleic acid combined with the solution containing a chaotropic material if needed. Impurity other than the nucleic acid which stuck to the carrier nonspecific by this is thoroughly washed from a nucleic acid-carrier complex, and last thing is possible.

[0021]The 2nd process in this invention is a process washed with the penetrant remover which is the purpose of removing a chaotropic material etc. and consists of low-salt concentration buffer solution the carrier to which ribonucleic acid stuck according to the 1st adsorption process. The penetrant remover which consists of low-salt concentration buffer solution here refers to the buffer solution which does not contain an organic solvent and chaotropic materials, such as ethanol, at all, and although tris system buffer solution is preferred as buffer solution, it is not limited in particular.

[0022]Low-salt concentration refers to the salt concentration of the grade which does not affect enzyme reactions, such as a RT-PCR reaction, when this buffer solution remains at the 3rd elution process, and mere water is also contained. In this invention, the buffer solution of 100 or less mM is preferred. This solution may contain a surface-active agent and pH in particular is not limited.

[0023]Washing here is operation of removing substances other than the nucleic acid unity solid phase to which ribonucleic acid stuck as much as possible from the mixture of living thing material, a solution, and nucleic acid unity solid phase, by contacting the carrier which ribonucleic acid combined to a penetrant remover, and dissociating again. As concrete separating mechanism in this invention, it changes with gestalten of the nucleic acid unity solid phase carrier to be used, and when a nucleic acid unity solid phase carrier is a gestalt of particles, centrifugal separation, filtration separation, column operation, etc. are preferred. If that in which the superparamagnetism metallic oxide was included is used as a carrier in particles, the simple magnetic separation using a magnet etc. becomes possible, and it is more suitable.

[0024]The 3rd process in this invention is an elution process. An elution process is a process which makes this ribonucleic acid elute from the nucleic acid unity solid phase to which ribonucleic acid stuck. Therefore, as an eluate used in this invention, if the elution of the ribonucleic acid from solid phase is promoted, it will not be limited in particular. For example, water or tris-EDTA buffer solution [10mM trischloride buffer solution, 1mM EDTA, pH 8.0] is preferred.

[0025]In this invention, it is required to promote elution with heating. If cooking temperature is a grade which does not have an adverse effect on ribonucleic acid, it will not be limited in particular, but its 50-70 ** is preferred. Cooking time is 30 seconds - a for [10 minutes] grade. Thus, the eluted ribonucleic acid can be directly used for the enzyme reaction which uses reverse transcriptase etc., without performing demineralization of dialysis, an ethanol sedimentation method, etc., and concentration operation.

[0026]One embodiment of this invention (a) ribonucleic acid in the included sample The guanidine salt of 4-7M, (b) Mix the neutral solution and nucleic acid unity solid phase carrier containing 0 to 5% of nonionic surfactant, EDTA of 0 - 0.2mM, and the reducing agent of 0-0.2M, make ribonucleic acid adsorb on a carrier, and rank second, The 1st penetrant remover containing the guanidine salt of 4-7M and 0 to 5% of nonionic surfactant washes a ribonucleic acid-carrier complex, (c) The 2nd penetrant remover that consists of water or low-salt concentration buffer solution of 100 or less mM washes a ribonucleic acid-carrier complex further, (d) After contacting the above-mentioned carrier at the last into the eluate which consists of water or low-salt concentration buffer solution of 100 or less mM, it is an extraction method of the ribonucleic acid making ribonucleic acid eluted in an eluate from the above-mentioned carrier by heating.

[0027]The reagent kit of this invention The guanidine salt of 4-7M, 0 to 5% of nonionic surfactant, EDTA of

0 - 0.2mM and the reducing agent of 0-0.2M, for example, the neutral solution containing 2-mercaptoethanol, The eluate which consists of the 2nd penetrant remover that consists of the 1st penetrant remover, the water, or the low-salt concentration buffer solution of 100 or less mM containing a nucleic acid unity solid phase carrier, the guanidine salt of 4-7M, and 0 to 5% of nonionic surfactant and water, or low-salt concentration buffer solution of 100 or less mM is included.

[0028]As mentioned above, the extraction method of the ribonucleic acid by this invention, Since water soluble organic solvents, such as ethanol which has a dangerous organic solvent and volatility, such as phenol, and inflammability, and acetone, are not needed at all and it comprises a simple step, It is clear that it can apply easily to the nucleic acid extracting apparatus which automated a ribonucleic acid extraction kit, the separating operation of solid phase, and reagent dispensation operation. the ribonucleic acid obtained by the method of this invention -- RT-PCR or NASBA (for example, EP0329822 item Description description) etc. -- it is usable as a mold of nucleic acid amplifying method.

[0029]There is the feature of this invention in using the low-salt concentration buffer solution which does not contain organic solvents, such as ethanol, at all as a penetrant remover of the nucleic acid unity solid phase carrier which ribonucleic acid combined. In order to prevent the elution from the carrier of the nucleic acid at the time of washing as such a penetrant remover conventionally, the water or low-salt concentration buffer solution which contains water soluble organic solvents, such as ethanol, 50 to 80% and which lowered the polarity of the solution is used. However, in this invention, since it is hard to elute ribonucleic acid from a solid phase carrier compared with a deoxyribonucleic acid even if it uses the low-salt concentration buffer solution which does not contain organic solvents, such as ethanol, at all as a penetrant remover, ribonucleic acid can be held on a solid phase carrier. Since this washing is eluted from a carrier, preparation of fewer ribonucleic acid samples of mixing of a deoxyribonucleic acid of a deoxyribonucleic acid is attained by it.

[0030]

[Example]Below, working example explains this invention concretely.

109 shares of preparation Escherichia coli JM of the extraction (1) Escherichia-coli sample of the ribonucleic acid from the sample containing working example 1 Escherichia coli -- LB agar medium (1% poly peptone.) It cultivated at 37 ** in a yeast extract and 1% sodium chloride 0.5% one whole day and night, next 37 ** of the single colony was cultivated in LB liquid medium for 12 hours. The phosphate buffer solution (PBS) (-) which collected Escherichia coli in centrifugality after measuring absorbance and OD660, and contained cow serum albumin (BSA) 7% With [137mM sodium chloride, 2.7mM potassium chloride, 4.3mM disodium hydrogenphosphate, and 1.4mM potassium dihydrogen phosphate (pH 7.4)], again, it was suspended and Escherichia coli was made into the sample so that absorbance and OD660 might be set to 0.5.

[0031](2) It is dissolution adsorption liquid of 700microl to the Escherichia coli sample prepared by the extraction above (1) of ribonucleic acid. [5.0M A guanidine thiocyanate, 2%TritonX-100, 25mM EDTA, 0.1M 2-mercaptoethanol and 50mM Tris-chloride buffer solution (pH 7.0)] was added and it dissolved. Magnetic silica prepared [ml] in 0.4g /to this (the particle diameter of 1-10 micrometers and 30% of tri-iron tetraoxide particles) [and] Specific-surface-area 280m^2/g, pore-volume 0.025 ml/g, surface pore diameters of 2-6 nm: 50microl addition of the suspension by the Suzuki fats-and-oils company was done, and it mixed with the vortex mixer for 10 minutes at the room temperature. Then, the micro tube was installed in the magnetic stand (MPC-M: made by a dynal company), magnetic silica particles were collected, and supernatant liquid was removed.

[0032]Next, after having removed the micro tube from the magnetic stand, adding the 1-ml penetrant remover I [6.5M Guanidine thiocyanate and 50mM tris-chloride (pH 6.4)] and fully mixing, particles were washed by installing in a magnetic stand and removing supernatant liquid similarly. After repeating this washing operation once again, it is the 1-ml penetrant remover II similarly. [5mM tris-chloride (pH 6.4)] washed particles twice. In the last, it is an eluate of 60microl to this. After adding [the sterilized water which carried out diethylpyrocarbonate processing] and suspending particles, it heated for 5 minutes at 65 **, and it installed in the magnetic stand, magnetic silica particles were collected, and supernatant liquid was collected. The amount of recovering liquid was about 50microl.

[0033](3) Extraction of nucleic acid (comparative example)

In order to compare the invention in this application with a conventional method, using the method of Boom

and others, the same sample was processed and nucleic acid was extracted.That is, the dissolution adsorption liquid [4.7M guanidine thiocyanate, 1.2%TritonX-100, 20mM EDTA, and 50mM tris-chloride buffer solution (pH 6.4)] of 900microl was added to the Escherichia coli sample prepared by (1), and it dissolved in it. 50microl addition of the magnetic silica (pore-volume 0.025 ml/g [1-10 micrometers, 30% of grain type particle / tri-iron tetraoxide / content, specific surface area 280m^2/g,] surface pore diameters of 2-6 nm: made by Suzuki fats-and-oils company) suspension prepared [ml] in 0.4g /to this was done, and it mixed for 10 minutes at the room temperature. Next, the micro tube was installed in the magnetic stand (MPC-M: made by a dynal company), magnetic silica particles were collected, and supernatant liquid was removed. Subsequently, the micro tube was removed from the magnetic stand, it washed by 1 ml of 70% ethanol, and acetone washed particles once twice with a 1-ml penetrant remover [5.3M Guanidine thiocyanate and 50mM tris-chloride (pH 6.4)]. After removing supernatant liquid, the micro tube was heated at 55 **, evaporative removal of the remaining acetone was carried out thoroughly, and particles were dried. In the last, it is an eluate of 100microl to this. After adding [the sterilized water which carried out diethylpyrocarbonate processing] and suspending particles, it heated for 10 minutes at 55 **, and it installed in the magnetic stand, magnetic silica particles were collected, and supernatant liquid was collected. The amount of recovering liquid was about 80microl.

[0034](4) nucleic acid solution 9mul extracted from Escherichia coli JM109 by the method of the analysis this invention method of the extracted nucleic acid or Boom and others by agarose gel electrophoresis, and coloring matter liquid (50% glycerol.) 0.25% bromophenol-blue 1microl was mixed, and agarose gel electrophoresis was presented 1% 1xTBE buffer solution performed migration for 100 V or 30 minutes. The dipping of the gel was carried out to the ethidium bromide solution for 15 minutes after the end of electrophoresis, and photography was performed under UV irradiation.

[0035]The result of the electrophoresis is shown in drawing 1. The nucleic acid extract with which the lane 1 was prepared among drawing 1 with the size marker (what digested lambda phage DNA with the restriction enzyme HindIII), and the lane 2 was prepared by this invention method, and the lane 3 show the zymogram of the nucleic acid extract prepared by the method of Boom and others. The extract obtained by this invention method has very little mixing of genomic DNA compared with the nucleic acid extract prepared by the method of Boom and others, and it turns out that the yield of RNA does not almost have a difference with the method of Boom and others so that clearly from drawing 1.

[0036]The hepatitis C patient's serum with which preparation of the detection (1) blood-serum sample by RT-PCR of working example 2 hepatitis-C-virus (HCV) RNA and HCV of the extraction 10^6 copy / ml of HCV-RNA are contained is diluted 10 times one by one using a normal negative blood serum, The dilution series of a 10 - 10^6 copy / ml was built, and these were made into extraction material. Blood serum sample 100microl (an equivalent for a 1 - 10^5 copy) of each dilution series was used, and RNA was extracted by the same method as working example 1.

[0037](2) Detection of HCV-RNA in recovering liquid was tried to the recovering liquid obtained by the amplification above (1) of HCV-RNA by RT-PCR by performing RT-PCR for the untranslation region of HCV-RNA at a target. RT-PCR is Hitoshi Okamoto's method. According to [J. Exp. Med., 60, and 215-222 (1990)], it carried out using a commercial reagent kit and RT-PCR high (made by Toyobo Co., Ltd.). First, the reagent for reverse transcription which contains M-MLV reverse transcriptase, the primer for reverse transcription, ribonucleotide inhibitor, and the buffer solution for a reaction in 5microl among the recovering liquid obtained above (1) was added, the last volume was set to 20microl, this was kept warm for 60 minutes 42 **, and the reverse transcription reaction was performed. Next, 2.5micro of reaction mixture I after a reverse transcription reaction is added to the reagent for PCR containing heat-resistant DNA polymerase, DNA after setting the last volume to 25microl 38 cycles of temperature cycles for [94 **] 30 seconds, for [53 **] 30 seconds, and for [72 **] 1 minute were carried out in thermal cycler (made by Perkin Elmer Cetus). Next, in addition to the reaction reagent for PCR which contains an inside primer further, for [94 **] 30 seconds, for [50 **] 1 minute, and for [72 **] 1 minute were carried out for 28 cycles amplification-products 1mul obtained at this reaction with the last volume I of 30micro, and two steps of PCR were performed.

[0038](3) 1micro of coloring matter liquid (50% glycerol, 0.25% bromophenol blue) I was mixed to detection PCR amplification product 9mul of the amplification products by agarose gel electrophoresis, and agarose gel electrophoresis was presented 1% 1xTBE buffer solution performed migration for 100 V or 20 minutes.

The dipping of the gel was carried out to the ethidium bromide solution for 15 minutes after the end of electrophoresis, and photography was performed under UV irradiation. The result a photograph of was taken is shown in drawing 2.

[0039]The lane 1 is a migration pattern of the RT-PCR amplification products of RNA by which extraction refining was carried out by the size marker which consists of a HincII digest of phichi174 phage DNA, and the method which the lanes 2-4 show to this example among drawing 2.

A result when the blood serum sample in which the lane 2 includes a $1x10^4$ copy, the lane 3 includes a $1x10^3$ copy, and the lane 4 contains HCV of a $1x10^2$ copy is used is shown.

It has checked that amplification products are seen about the blood serum sample which contains HCV of a 10^3 copy from drawing 2, and extraction of HCV-RNA was possible and it could be promptly used for the analysis by RT-PCR from a blood serum sample by the method of this invention.

[0040]

[Effect of the Invention]according to this invention method -- the quickness from living thing materials, such as a blood serum and plasma, -- extraction of ribonucleic acid is attained simple and safely. The ribonucleic acid extracted with this extraction method can be promptly used for nucleic acid amplifying method, such as RT-PCR. In this extraction method, for a being [it / necessary / to use no organic solvent and to carry out stoving of the carrier] reason, compared with a conventional method, it is safe and simple, and the time which extracting processing takes can also be shortened substantially, and the positive high result of reproducibility is obtained.

[Translation done.]

DESCRIPTION OF DRAWINGS

[Brief Description of the Drawings]
[Drawing 1]It is a photograph replaced with the Drawings in which the agarose-gel-electrophoresis pattern of the ribonucleic acid extracted from the Escherichia coli sample is shown by the method of this invention, and the method of Boom and others.
[Drawing 2]It is a photograph replaced with the Drawings in which the agarose-gel-electrophoresis result of the amplification products which amplified the ribonucleic acid extracted from the HCV positive blood serum by the method of this invention by RT-PCR is shown.

[Translation done.]